

1/23

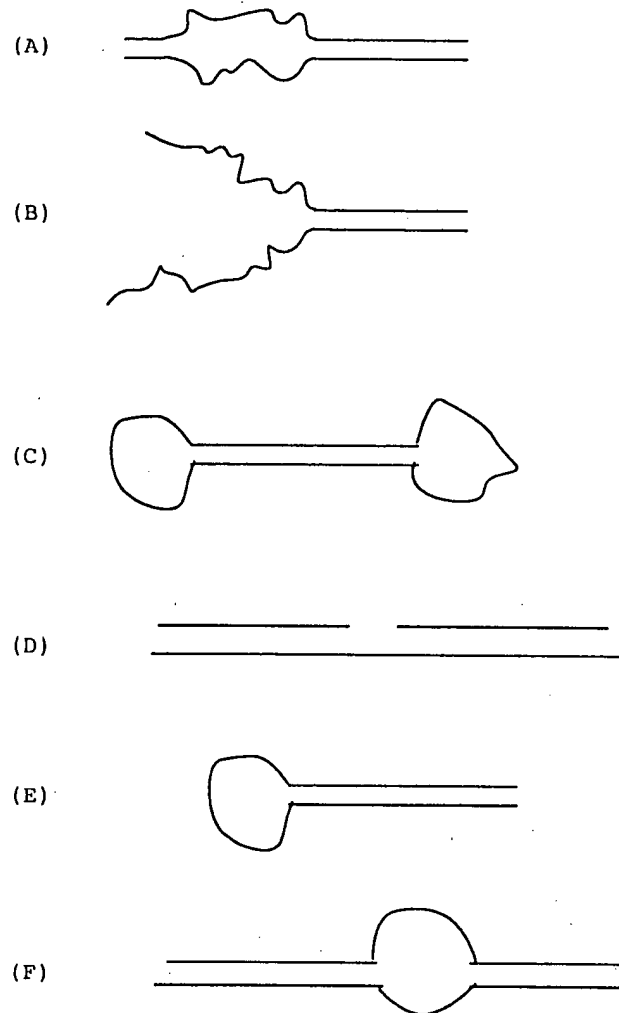


Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region

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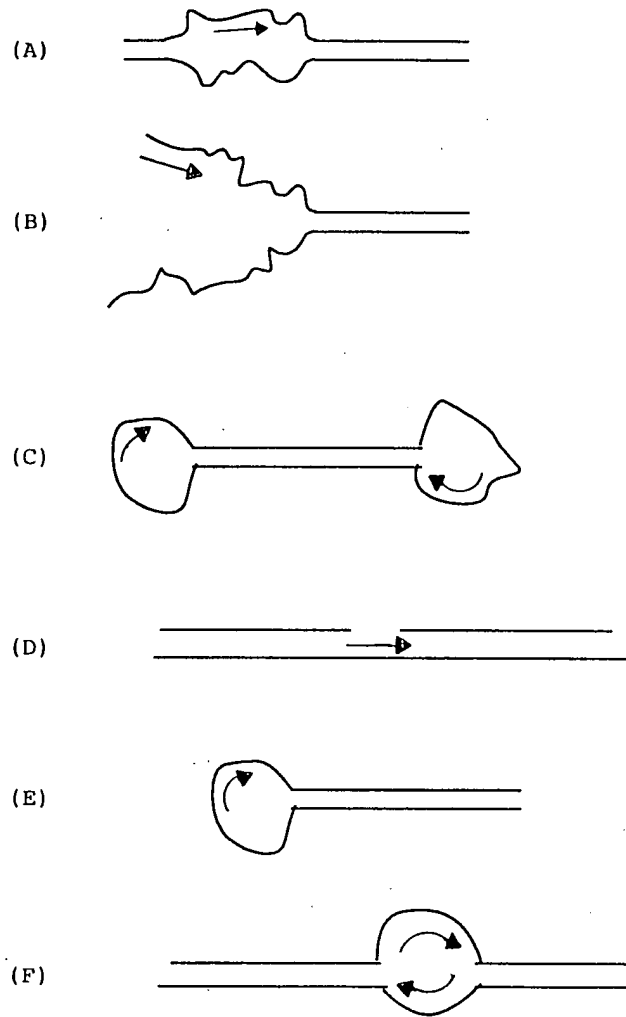


Figure 2 (A-F)

Functional Forms of the Construct

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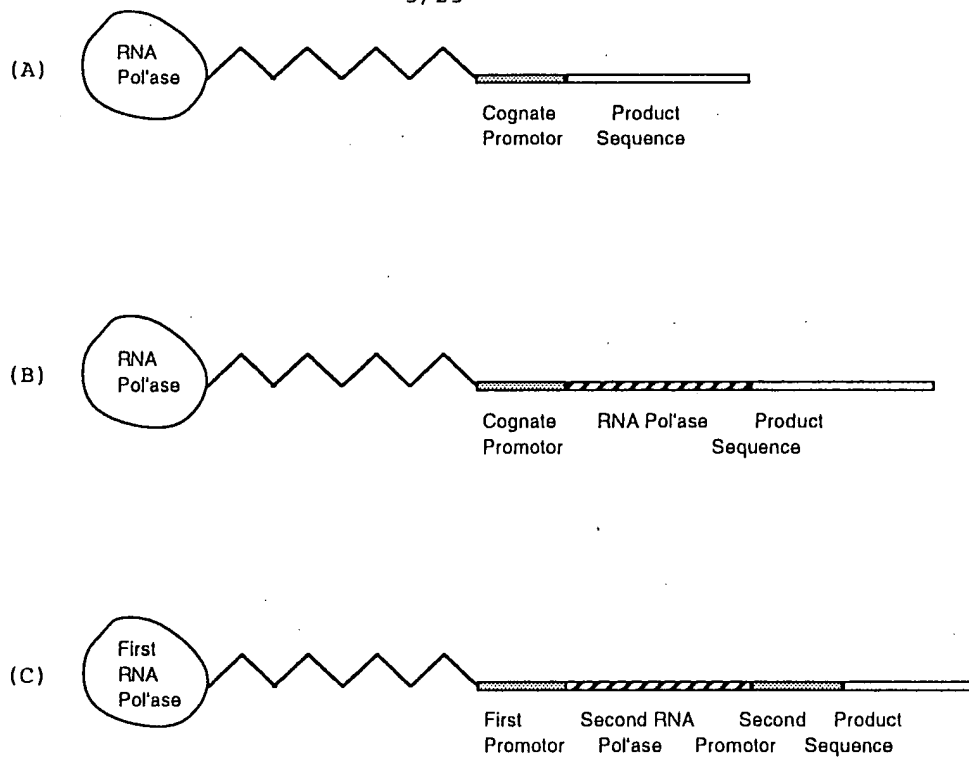


Figure 3 (A-C)

Three Constructs with an RNA Polymerase
Covalently Attached to a Transcribing Cassette

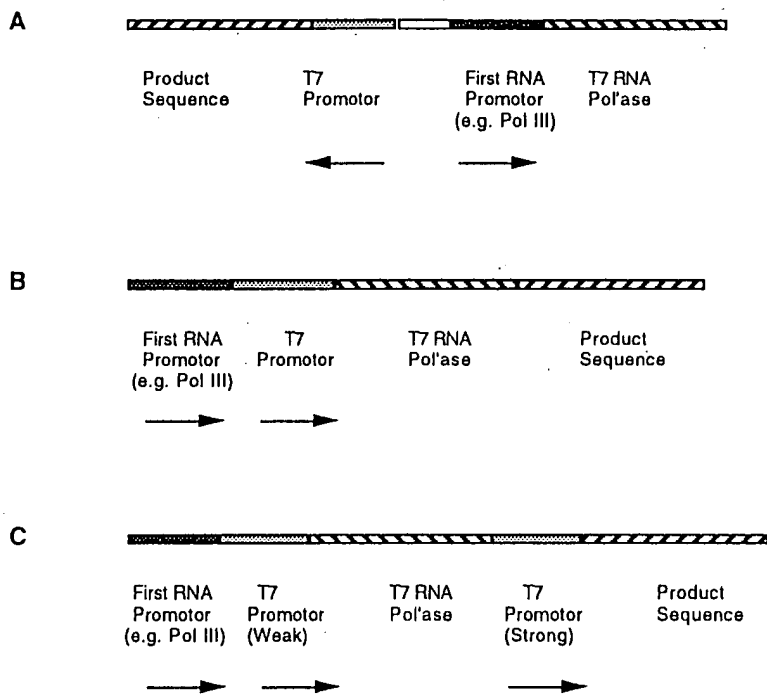


Figure 4 (A-C)

Three Constructs with Promoters for Endogenous RNA Polymerase

M13mp18. Seq Length: 7250

1.	AATGCTACTA	CTATTAGTAG	AATTGATGOC	ACCTTTTCAG	CTOGGCGOCC
51.	AAATGAAAAT	ATAGCTAAAC	AGGTTATTGA	CCATTTGCGA	AATGTATCTA
101.	ATGGTCAAAC	TAAATCTACT	CGTTGCGAGA	ATTGGGAATC	AACTGTTACA
151.	TGGAATGAAA	CTTCAGACA	COGTACTTTA	GTTGCATATT	TAAAACATGT
201	TGAGCTACAG	CAOCAGATTC	AGCAATTAAG	CTCTAAGOCA	TCOGCAAAAA
251	TGAOCTCTTA	TCAAAAGGAG	CAATTAAAGG	TACTCTCTAA	TOCTGAOCTG
301.	TTGGAGTTTG	CTTCGGTCT	GGTTGCTTT	GAAGCTCGAA	TTAAAACGGG
351.	ATATTTGAAG	TCTTTOGGGC	TTOCTCTTAA	TCTTTTGTAT	GCAATTOGCT
401.	TTGCTTCTGA	CTATAATAGT	CAGGGTAAAG	ACCTGATTTT	TGATTTATGG
451.	TCATTCTCGT	TTTCTGAACT	GTTTAAAGCA	TTTGAGGGGG	ATTCAATGAA
501.	TATTTATGAC	GATTOGCGAG	TATTGGAOGC	TATOCAGTCT	AAACATTTTA
551.	CTATTACOC	CTCTGGCAA	ACTTCTTTTG	CAAAGOCTC	TCGCTATTTT
601.	GGTTTTTATC	GTCGTCTGGT	AAACGAGGGT	TATGATAGTG	TTGCTCTTAC
651.	TATGOCTOGT	AATTCCTTTT	GGGTTATGT	ATCTGCATTA	GTTGAATGTG
701.	GTATTOCTAA	ATCTCAACTG	ATGAATCTTT	CTAOCGTAA	TAATGTTGTT
751.	COGTTAGITC	GTTTTATTAA	CGTAGATTTT	TCTTCCCAAC	GTOCTGACTG
801.	GTATAATGAG	CCAGTTCTTA	AAATGCGATA	AGGTAATTCA	CAATGATTAA
851.	AGTTGAAATT	AAOCATCTC	AAGCCCAATT	TACTACTOGT	TCTGGTGTTC
901.	TOGTCAGGGC	AAGCTTATT	CACTGAATGA	GCAGCTTTGT	TACGTTGATT
951.	TGGGTAATGA	ATATCOGGTT	CTTGTOGAAG	ATTACTCTTG	ATGAAGGTCA
1001	GOCAGOCAT	GCGOCTGGTC	TGTACACOGT	TCATCTGTCC	TCTTTCAAAG
1051	TTGGTCAGTT	CGGTTCCCTT	ATGATTGAOC	GTCTGCGOCT	CGTTCCGGCT
1101	AAGTAACATG	GAGCAGGTGG	CGGATTTGGA	CACAATTTAT	CAGGCGATGA
1151	TACAAATCTC	CGTTGTAOCTT	TGTTTGGGCG	TTGGTATAAT	CGCTGGGGGT
1201	CAAAGATGAG	TGTTTTAGTG	TATTCTTTGG	CCTCTTTOGT	TTTAGGTTGG

Figure 5

M13mp18 Nucleic Acid Sequence

1251	TGCTTGGTA	GTGGCATTAC	GTATTTTACC	CGTTTAATGG	AACTTCTCTC
1301	ATGAAAAAGT	CTTTAGTCCT	CAAAGCCTCT	GTAGCGGTG	CTAOCCTCGT
1351	TOOGATGCTG	TCTTTOGCTG	CTGAGGGTGA	CGATCOOGCA	AAAGCGGCGT
1401	TTAACTCOCT	GCAAGCCTCA	GCGAOCGAAT	ATATCGGTTA	TGCTGCGCGG
1451	ATGGTTGTTG	TCATTGTGGG	CGCAACTATC	GGTATCAAGC	TGTTTAAGAA
1501	ATTCACTCG	AAAGCAAGCT	GATAAACCGA	TACAATTAAA	GGCTCTTTT
1551	GGAGCCTTTT	TTTTTGAGA	TTTCAACGT	GAAAAATTA	TTATTOGCAA
1601	TTCTTTAGT	TGTTCTTTC	TATTCTCACT	CGCTGAAAC	TGTTGAAAGT
1651	TGTTTAGCAA	AACCCATAC	AGAAAATCA	TTACTAACG	TCTGGAAGA
1701	CGACAAAAC	TTAGATCGTT	ACGCTAACTA	TGAGGGTTGT	CTGTGGAATG
1751	CTACAGGCGT	TGTAGTTTGT	ACTGGTGAOG	AACTCAGTG	TTACGGTACA
1801	TGGGTTCTA	TTGGGCTTGC	TATCOCTGAA	AATGAGGGTG	GTGGCTCTGA
1851	GGGTGGGGT	TCTGAGGGTG	GCGTTCTGA	GGGTGGGGT	ACTAAOCTC
1901	CTGAGTACGG	TGATACAOC	ATTOCGGGCT	ATACTTATAT	CAOCCCTCTC
1951	GACGGCACTT	ATCOGCTGG	TACTGAGCAA	AACCGCTA	ATCTAATCC
2001	TTCTCTTGAG	GAGTCTCAGC	CTCTTAATAC	TTTCATGTTT	CAGAATAATA
2051	GGTTOGAAA	TAGGCAGGGG	GCATTAAC	TTTATAOCC	CACTGTTACT
2101	CAAGGCACTG	AACCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGOCATG	TATGACGCTT	ACTGGAACGG	TAAATTCAGA	GACTGCGCTT
2201	CAAGGCACTG	AACCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGOCATG	TGCTCAAOC	TOCTGTCAAT	GCTGGCGGGG	GCTCTGGTGG
2201	TOCATCTGG	CTTTAATCAA	GATOCATTGG	TTTGTGAATA	TCAAGGOCOA
2251	TGCTCTGAOC	TGCTCAAOC	TOCTGTCAAT	GCTGGCGGGG	GCTCTGGTGG
2301	TGGTTCTGGT	GGGGCTCTG	AGGGTGGTGG	CTCTGAGGGT	GGGGTTCTG
2351	AGGGTGGGG	CTCTGAGGGA	GGGGTTCCG	GTGGTGGCTC	TGGTTCCGGT
2401	GATTTTGATT	ATGAAAAGAT	GGCAAAOGCT	AATAAGGGGG	CTATGAOCGA
2451	AAATGOCGAT	GAAAAOGGG	TACAGTCTGA	CGCTAAAGGC	AACTTGATT

Figure 5

M13mp18 Nucleic Acid Sequence

2501	CTGTGCTAC	TGATTACGGT	GCTGCTATCG	ATGGTTTCAT	TGGTGAOGTT
2551	TOCGGOCCTG	CTAATGGTAA	TGGTGCTACT	GGTGATTTTG	CTGGCTCTAA
2601	TTCCAAATG	GCTCAAGTOG	GTGAOGGTGA	TAATCAOCT	TTAATGAATA
2651	ATTTOOGTCA	ATATTTACCT	TOOCTOCTC	AATOGGTTGA	ATGTGGOOCT
2701	TTTGCTTTA	GOGCTGGTAA	AOCATATGAA	TTTTCTATTG	ATTGTGACAA
2751	AATAAACTTA	TTOOGTGGTG	TCTTTGCGTT	TCTTTTATAT	GTTGOCACCT
2801	TTATGTATGT	ATTTTCTACG	TTTGCTAACA	TACTGCGTAA	TAAGGAGTCT
2851	TTATCATGCC	AGTTCCTTTG	GGTATTCOGT	TATTATTGCG	TTTOCTCGGT
2901	TTCTTCTGG	TAACCTTGTT	CGGCTATCTG	CTTACTTTTC	TTAAAAAGGG
2951	CTTOGGTAAG	ATAGCTATTG	CTATTTCAAT	GTTTCTTGCT	CTTATTATTG
3001	GGCTTAACTC	AATTCTTGTTG	GGTTATCTCT	CTGATATTAG	CGCTCAATTA
3051	COCTCTGACT	TTGTTCAAGG	TGTTCAAGTTA	ATTCTCOOCT	CTAATGOGCT
3101	TCOCTGTTTT	TATGTTATTC	TCTCTGTAAA	GGCTGCTATT	TTCAATTTTG
3151	ACGTAAACA	AAAAATOGTT	TCTTATTTGG	ATTGGGATAA	ATAATATGGC
3201	TGTTTTATTT	GTAACCTGCA	AATTAGGCTC	TGGAAAGAOG	CTOGTTAGOG
3251	TTGGTAAGAT	TCAGGATAAA	ATTGTAGCTG	GGTGCAAAAT	AGCAACTAAT
3301	CTTGATTTAA	GGCTTCAAAA	OCTOOCGCAA	GTOGGGAGGT	TOGCTAAAAC
3351	GCTOGOGITT	CTTAGAATAC	OGGATAAGOC	TTCTATATCT	GATTTGCTTG
3401	CTATTGGGOG	OGGTAATGAT	TOCTACGAATG	AAAATAAAAA	CGGCTTGCTT
3451	GTTCTOGATG	AGTGOGGTAC	TTGGTTTAAT	AOCOCTTCTT	GGAATGATAA
3501	GGAAAGACAG	COGATTATTG	ATTGGTTTCT	ACTGCTCGT	AAATTAGGAT
3551	GGGATATTAT	TTTTCTTGTT	CAGGACTTAT	CTATTGTTGA	TAAACAGGOG
3601	OGTTCTGCAT	TAGCTGAACA	TGTTGTTTAT	TGTOGTGTC	TGGACAGAAT
3651	TACTTTACCT	TTTGTOGGTA	CTTTATATTC	TCTTATTAAT	GGCTOGAAAA
3701	TGCTCTGOC	TAAATTACAT	GTTGGOGTTG	TTAAATATGG	OGATTCTCAA
3751	TTAAGOOCTA	CTGTTGAGOG	TTGGCTTTAT	ACTGGTAAGA	ATTTGTATAA
3801	CGCATATGAT	ACTAACAGG	CTTTTCTAG	TAATTATGAT	TCOGGTGTTT

Figure 5

M13mp18 Nucleic Acid Sequence

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3851 ATTCTTATTT AAGGCTTAT TTATCACACG GTOGGTATTT CAAACCATTA
3901 AATTTAGGTC AGAAGATGAA ATTAACATAA ATAATATTGA AAAAGTTTTT
3951 TCGGTTCTTT TGTCTTGCGA TTGGATTTGC ATCAGCATTT ACATATAGTT
4001 ATATAACCCA AOCTAAGGCG GAGGTTAAAA AGGTAGTCTC TCAGAOCTAT
4051 GATTTTGATA AATTCATCTAT TGAATCTTCT CAGGCTCTTA ATCTAAGCTA
4101 TCGCTATGTT TTCAAGGATT CTAAGGGAAA ATTAATTAAT AGOGAOGATT
4151 TACAGAAGCA AGGTTATTCA CTCACATATA TTGATTTATG TACTGTTTCC
4201 ATTAATAAAG GTAATTCAAA TGAAATTGTT AAATGTAATT AATTTTGTTT
4251 TCTTGATGTT TGTTTCATCA TCTTCTTTTG CTCAGGTAAT TGAAATGAAT
4301 AATTOGCTC TCGGCGATTT TGTAACCTGG TATTCAAAGC AATCAGGCGA
4351 AATCGGTTATT GTTCTCTCCG ATGTAAAAGG TACTGTTACT GTATATTCAT
4401 CTGAAGTTAA AOCTGAAAAT CTAAGCAATT TCTTTATTTT TGTTTACGT
4451 GCTAATAATT TTGATAATGGT TGGTTCAATT CCTTCATAA TTCAGAAGTA
4501 TAATCCAAAC AATCAGGATT ATATTGATGA ATTGOCATCA TCTGATAATC
4551 AGGAATATGA TGATAATTCC GCTCCTCTG GTGGTTTCTT TGTTCCGCA
4601 AATGATAATG TTAATCAAAC TTTTAAATTT AATAAGTTC GGGCAAAGGA
4651 TTAATAACGA GTTGTOGAAT TGTTTGTAAG GTCTAATACT TCTAAATCCT
4701 CAAATGTATT ATCTATTGAC GGCTCTAATC TATTAGTTGT TAGTGCTCCT
4751 AAAGATATTT TAGATAACCT TCCTCAATTC CTTTCTACTG TTGATTTGCC
4801 AACTGAOCAG ATATTGATTG AGGGTTTGAT ATTTGAGGTT CAGCAAGGTG
4851 ATGCTTTAGA TTTTTCATTT GCTGCTGGCT CTCAGGTTGG CACTGTTGCA
4901 GGGGGTGTTA ATACTGAACG CCTCACTCT GTTTTATCTT CTGCTGGTGG
4951 TTOGTTGGGT ATTTTAAATG GCGATGTTTT AGGGCTATCA GTTCCGGCAT
5001 TAAAGACTAA TAGOCATTCA AAAATATTGT CTGTGCCAAG TATTCTTACG
5051 CTTTCAGGTC AGAAGGGTTC TATCTCTGTT GGCCAGAATG TCCCTTTTAT
5101 TAAAGACTAA TAGOCATTCA AAAATATTGT CTGTGCCAAG TATTCTTACG
5151 CGATTGAGCG TCAAAATGTA GGTATTTCCA TGAGCGTTTT TCCTGTTGCA

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Figure 5

M13mp18 Nucleic Acid Sequence


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5201 ATGGCTGGGG GTAATATTGT TCTGGATATT AOCAGCAAGG OOGATAGTTT
5251 GAGTTCCTCT ACTCAGGCAA GTGATGTTAT TACTAATCAA AGAAGTATTG
5301 CTACAAOOGT TAATTTGOGT GATGGACAGA CTCTTTTACT OGGTGGOOCTC
5351 ACTGATTATA AAAACACTTC TCAAGATTCT GGOGTAOOGT TOCTGTCTAA
5401 AATCOCTTTA ATOGGOCTOC TGTTTAGCTC OOGCTCTGAT TOCAAOGAGG
5451 AAAGCAOGTT ATAOGTGCTC GTCAAAGCAA CCATAGTACG OGCOCTGTAG
5501 OGGOGCATTa AGOGOGGOGG GTGTGGTGGT TAOGOGCAGC GTGAOOGCTA
5551 CACTTGOCAG OGCOCTAGOG COOGCTOCTT TCGCTTTCTT COCTTCTTTT
5601 CTGOCACOGT TOGOOGGCTT TCOOOGTCAA GCTCTAAATC GGGGGCTOOC
5651 TTTAGGGTTC OGATTTAGTG CTTTACGGCA OCTOGAOCOC AAAAAACTTG
5701 ATTTGGGTGA TGGTTCAOGT AGTGGGOCAT OGCOCTGATA GACGGTTTTT
5751 OGCOCTTTGA OGTTGGAGTC CAOGTTCTTT AATAGTGGAC TCTTGTTOCA
5801 AACTGGAACA ACACTCAOOC CTATCTOGGG CTATTCTTTT GATTTATAAG
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6001 GGGGOCOAAT AOGCAAAOCG OCTCTOOCOG OGOGTTGGOC GATTCATTAA
6051 TGCAGCTGGC AOGACAGGTT TCOOGACTGG AAAGOGGGCA GTGAGOGCAA
6101 OGCAATTAAT GTGAGTTAGC TCACTCATTa GGCAOOCOCG GCTTTACACT
6151 TTATGCTTCC GGCTOGTATG TTGTGTGGAA TTGTGAGOGG ATAACAATTT
6201 CACACAGGAA ACAGCTATGA CCATGATTAC GAATTGAGC TOGGTAOCOG
6251 GOGATCTCT AGAGTOGAOC TGCAGGCATG CAAGCTTGGC ACTGGCOGTC
6301 GTTTTACAAC GTOGTGACTG GGAAAACOC TGGGTTAOC AACTTAATOG
6351 OCTTGACGCA CAATCOOCTT TOGOCAGCTG GOGTAATAGC GAAGAGBOOC
6401 GCACOGATOG COCTTCCAA CAGTTGOGCA GOCTGAATGG CGAATGGOGC
6451 TTTGCTGGT TTOGGCAOC AGAAGOGGTG OOGGAAAGCT GGCTGGAGTG
6501 OGATCTTOCT GAGGCOGATA OGGTGTGTGT COOCTCAAAC TGGCAGATGC

```

Figure 5

M13mp18 Nucleic Acid Sequence

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6551	ACGGTTAOGA	TGOGGOCATC	TACACCAAOG	TAAOCTATOC	CATTACGGTC
6601	AATCOGGOGT	TTGTTCCAC	GGAGAATCOG	ACGGGTGTGT	ACTOGCTCAC
6651	ATTTAATGTT	GATGAAAGCT	GGCTACAGGA	AGGOCAGAOG	CGAATTATTT
6701	TTGATGGOGT	TOCTATTGGT	TAAAAAATGA	GCTGATTTAA	CAAAAATTTA
6751	ACGCGAATTT	TAACAAAATA	TTAACGTTTA	CAATTTAAAT	ATTTGCTTAT
6801	ACAATCTTCC	TGTTTTTGGG	GCTTTTCTGA	TTATCAACCG	GGGTACATAT
6851	GATTGACATG	CTAGTTTTAC	GATTACCGTT	CATCGATTCT	CTTGTTTGCT
6901	CCAGACTCTC	AGGCAATGAC	CTGATAGCCT	TTGTAGATCT	CTCAAAAATA
6951	GCTACOCCTCT	COGGCATGAA	TTTATCAGCT	AGAACGGTTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	COGGCCTTTC	TCACOCCTTTT	GAATCTTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAAA	TATATGAGGG	TTCTAAAAAT
7101	TTTTATCCTT	GCGTTGAAAT	AAAGGCTTCT	COOGCAAAAG	TATTACAGGG
7151	TCATAATGTT	TTTGGTACAA	COGATTTAGC	TTTATGCTCT	GAGGCTTTAT

Figure 5

M13mp18 Nucleic Acid Sequence

COMPLEMENTARY TO M₁₃

POSITION	5' . . . 3'	POSITION	
645	AGCAACACTATCATA	631	M ₁₃ /1
615	ACGACGATAAAAAAC	601	M ₁₃ /2
585	TTTTCGAAAAGAAGT	571	M ₁₃ /3
555	AATAGTAAAATGTTT	541	M ₁₃ /4
525	CAATACTGCGGAATG	511	M ₁₃ /5
495	TGAATCCCCCTCAAA	481	M ₁₃ /6
465	AGAAAACGAGAATGA	451	M ₁₃ /7
435	CAGGTCTTTACCGTG	421	M ₁₃ /8
405	AGGAAAGCGGATTGC	391	M ₁₃ /9
375	AGGAAGCCCGAAAGA	361	M ₁₃ /10

COMPLEMENTARY TO SS PHAGE DNA

POSITION	5' . . . 3'	POSITION	
351	ATATTTGAAGTCTTT	366	M ₁₃ /11
371	TCTTTTGTGCAAT	386	M ₁₃ /12
391	CTATAATACTCAGGG	406	M ₁₃ /13
411	TGATTATGGTCATT	426	M ₁₃ /14
431	GTTTAAAGCATTGGA	446	M ₁₃ /15
451	TATTTATGACGATTC	466	M ₁₃ /16
471	TATCCAGTCTAAACA	486	M ₁₃ /17
491	CTCTGGCAAACTTC	506	M ₁₃ /18
511	TCGCTATTTTGGTTT	526	M ₁₃ /19
531	AAACGAGGGTTATGA	546	M ₁₃ /20

Figure 6

Primers for Nucleic Acid Production
Derived from M13mp18 Sequence

12/23

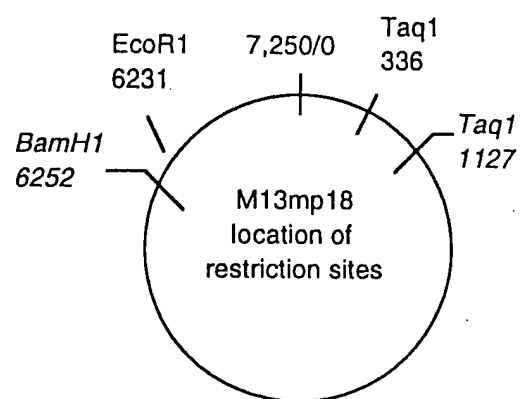


Figure 7

Appropriate M13mp18 Restriction Sites

13/23



Lane 1: from calf thymus + Taq digested mp18 amplification reaction
Lane 2: from Taq digested mp18 amplification reaction
Lane 3: from calf thymus amplification reaction
Lane 4: øX174 Hinf1 size marker

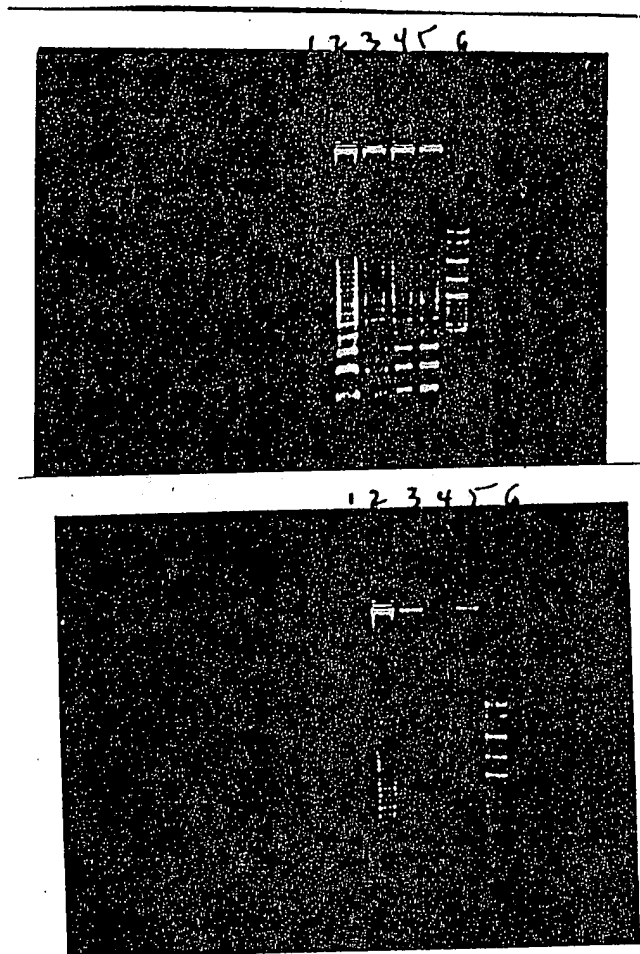
Figure 8

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Lane 1: no template
Lane 2: mp18 template, phosphate buffer
Lane 3: MspI/pBR322 size marker
Lane 4: mp18 template, MOPS buffer

Figure 9

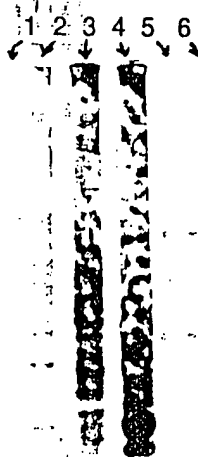


Top= (+) Template
 Bottom= (-) Template

Lane 1: phosphate buffer
 Lane 2: MES
 Lane 3: MOPS
 Lane 4: DMAB
 Lane 5: DMG
 Lane 6: pBR322/Mspl size marker

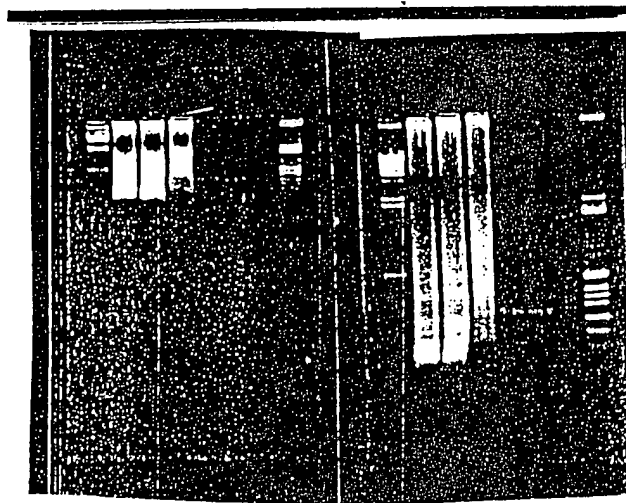
Figure 10

16/23



Lane 1: DMAB buffer, no template
Lane 2: DMAB buffer, mp18 template
Lane 3: DMG buffer, no template
Lane 4: DMG buffer, mp18 template
Lane 5: No reaction
Lane 6: 200 ng Taq I digested mp18
size marker/positive control

Figure 11



First Time Interval Second Time Interval

Agarose Gel Analysis

- Lane 1: lambda Hind III marker
- Lane 2: Amp/Untreated
- Lane 3: Amp/Kinased
- Lane 4: Amp/Kinased/Ligated
- Lane 5: PCR/Untreated
- Lane 6: PCR/Kinased
- Lane 7: PCR/Kinased/Ligated
- Lane 8: ϕ X174/Hinf1 marker

Figure 12

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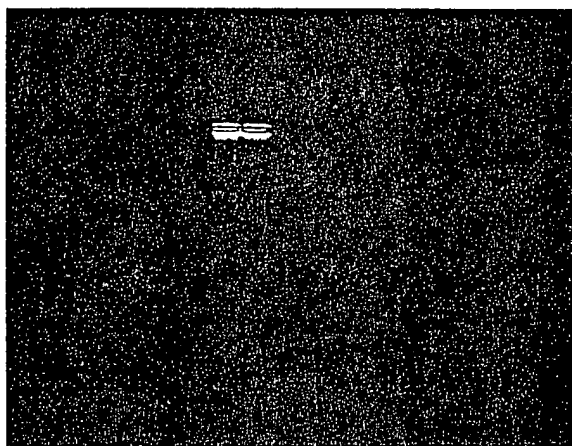
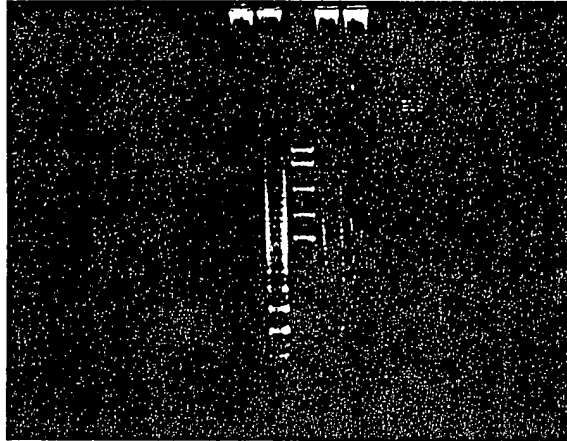


Figure 13

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1 2 3 4 5 6



Lane 1: Primers alone

Lane 2: Primers + taq digested M13 DNA

Lane 3: Molecular weight markers

Lane 4: Primers + RNA

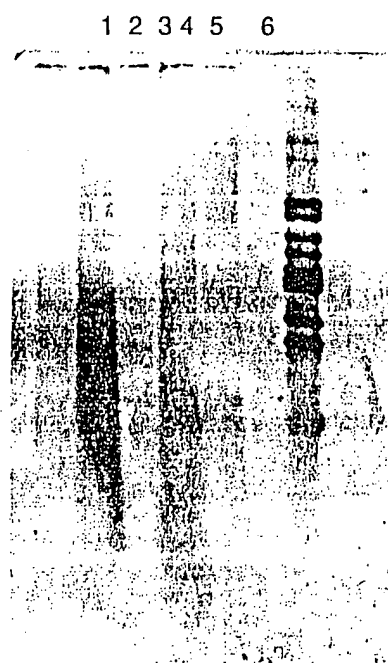
Lane 5: Primers alone

Lane 6: M13 digested DNA

Buffer was dimethyl amino glycine, pH 8.6

Figure 14

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Lane 1: Primers alone

Lane 2: Primers + taq digested M13 DNA

Lane 3: Molecular weight markers

Lane 4: Primers + RNA

Lane 5: Primers alone

Lane 6: M13 digested DNA

Buffer was dimethyl amino glycine, pH 8.6

Figure 15

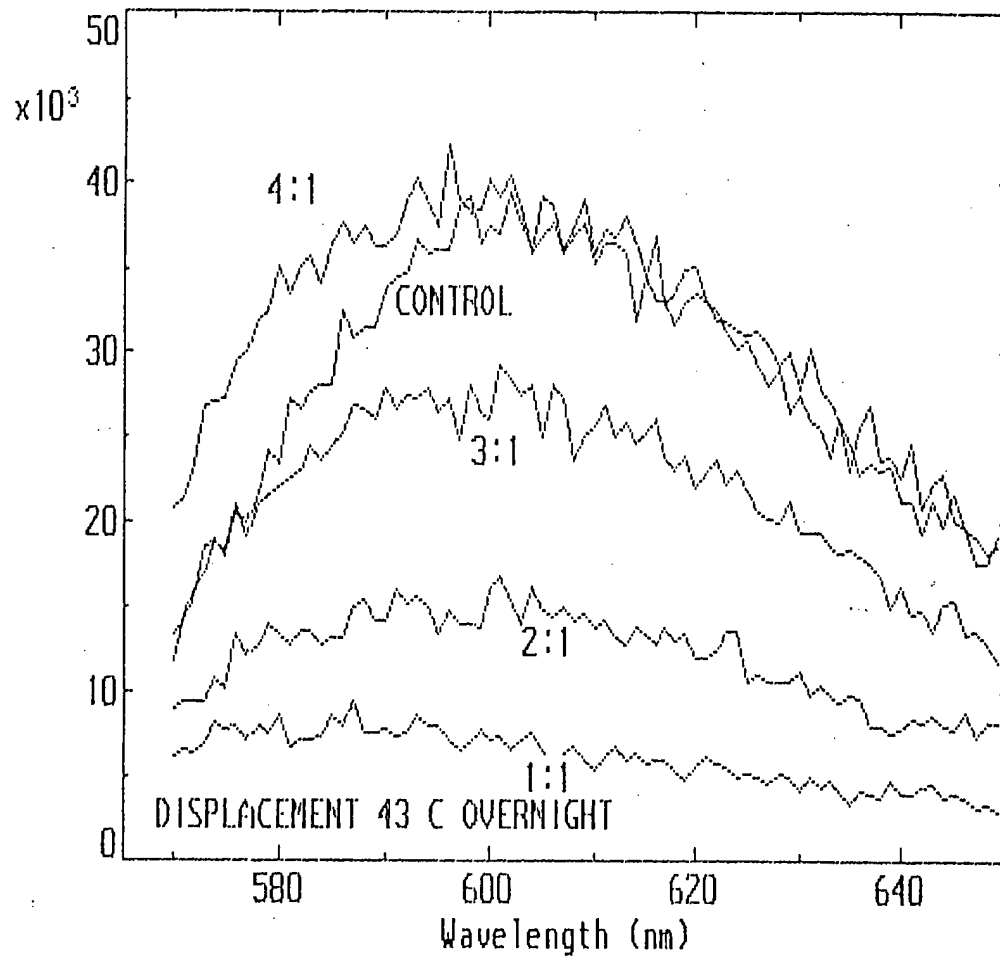


Figure 16

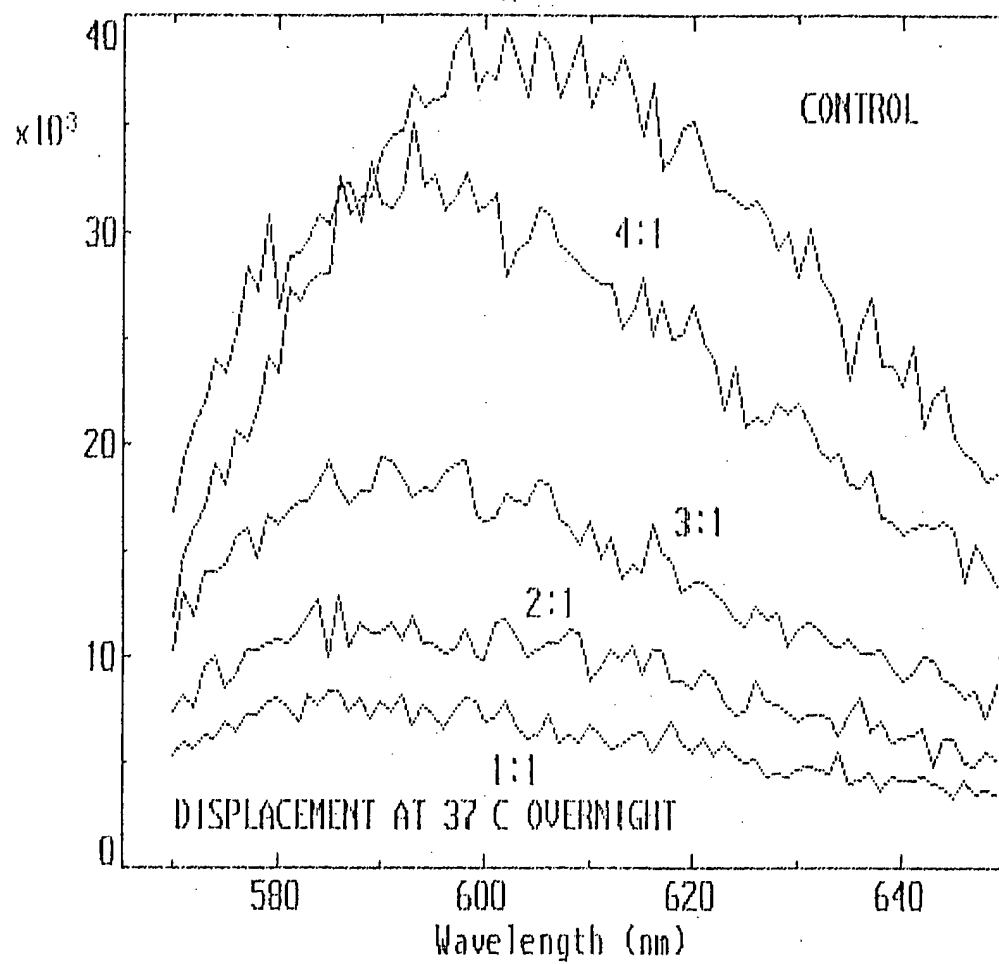


Figure 17

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pIBI 31-BH5-2

fmet AUG of Lac z (T7 Promotor region....
LAC PROMOTOR..ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA
oligo 50-mer 3'- tac t'aa t'gc ggt' ct'a t'ag t'Vt aat' tat' gct' gag t'ga t'at' c-5'
10 base insert
T7 RNA Start («« T3 Promotor Region)
IGGG CTC ICCT TTA GTG ACG GTT AAT
....») «- T3 Start Signal

pIBI 31 BSII/HCV

fmet AUG of Lac z (T3 Promotor region -») T3 RNA Start
LAC PROMOTOR ..ATG ACC ATG ATT ACG CCA AGC TCG AAA TTA ACC CTC ACT AAA /GGG
oligo 50-mer 3'- tac t'aa t'ac t'aa t'gc ggt' t'V--10 base insert--.....
MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....
«- T7 Promotor Region)
«- T7 Start Signal
5'-ct'a t'ag t'ga gt'c gt'a tt'a at'.....

Figure 18